

1/44

09/508377-



FIGURE 1

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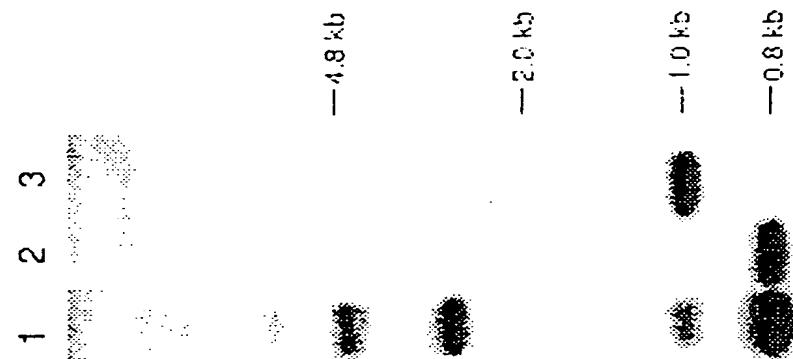


FIGURE 2

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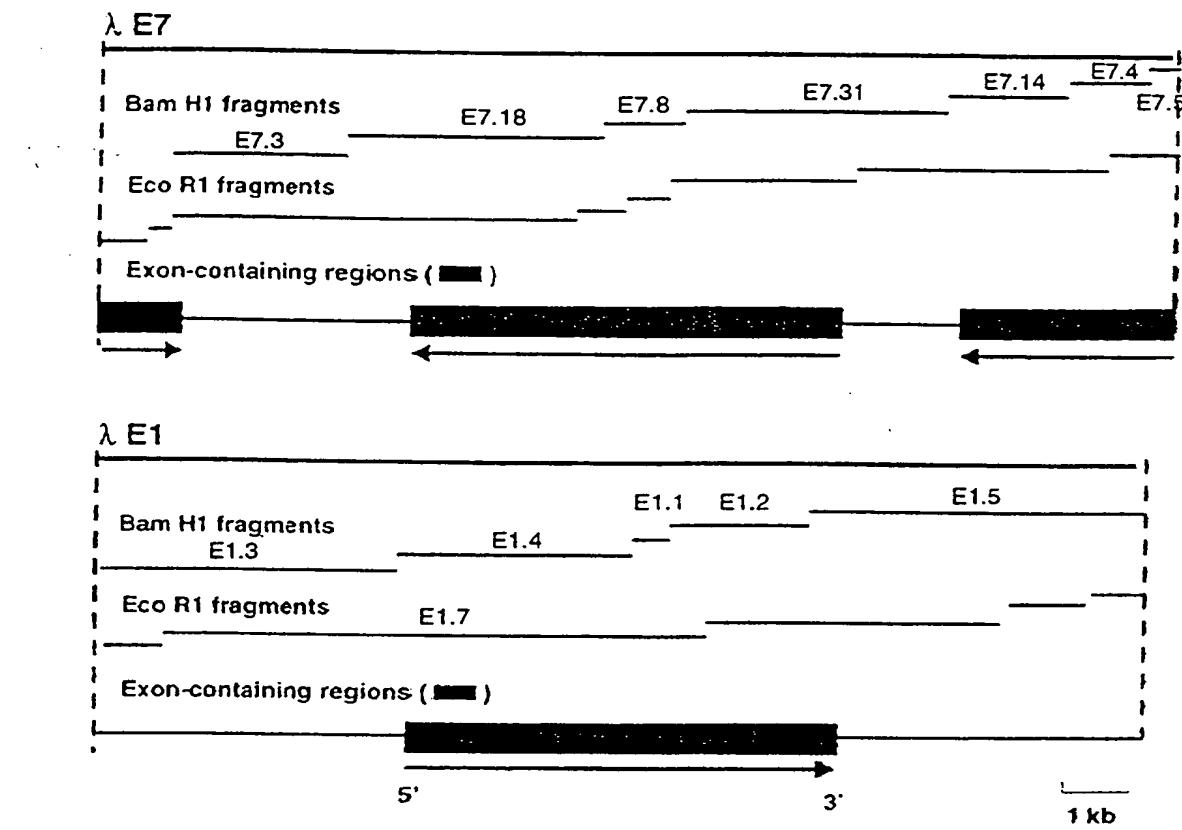


FIGURE 3

	1	50
RSBEI	.....	*****pl lp***** **ag*****
MSBEI	.....	*****p** tpi p***r ***h***aa pg*****
D4cDNA	.....	*****ap*c **sl.***p **pa***g **s*.....
PESBEII	.....	.....
POSBE	meinfkvlsk	pirgsfp*f* pkv*sgas*n kic*psqh*t *lkf*sqers
D2cDNA	.....	*****s**11 prp*a*.... .*****1* *****ggk
Consensus	-----	-MLCLTSSSS SP-S-APPR- SRS-ADRPS? GIIAGGNVR
	51	100
RSBEI	1..***v*...	*p*****g** *tn***pa** rk***v*vv ***.*****
MSBEI	1..**l**qc	ka***gv*** ***ataa*v q*d****ak g***.*****
D4cDNA	.....	*****p*s* prdy****a* *g*..gd***
PESBEII	.....	.....mt d*ks**psv **f..*nig*
POSBE	w..d*s*t*k	*rv*kde*mk h*saisa*lt d***s***pl* ***kt*nigl
D2cDNA	rlsv*p***f	11***l****a ***sf*s*** rg***ia*** tgygs*****
Consensus	---SV-SVP-	S-RRSWPRKV KSKFSV-VTA -DNKTMAT- EDV--DHLPI
	101	150
RSBEI	*****e*	*****n***i** *****c***** *****.*****v
MSBEI	*****i*	*****gs**e n**s**s*** *****.*****n
D4cDNA	*****ag*	*****s*****k *****s*** *****.*****
PESBEII	lnv**ss**p*	*****k***** **h**k***e y***q**a* *****f*r*
POSBE	ln***t**p*	1*****h***** v***m***** y***p***aq *****f*r*
D2cDNA	*****l**ae*	*****d*trn* i*****s***** *****s*****
Consensus	YDLDPKLE-F	KDHFRYRMKR YLDQKHLIEK HEGGLEEFSK GYLKFGINTE
	151	200
RSBEI	*g*****	*****ak* *****k***** **k*****
MSBEI	*dg*****	*****e*** ***d***a** *****k***** **k*d**k**
D4cDNA	nd*****	***m***** *****g* r*t**n***** *****.*****
PESBEII	*dgis*****	*****i** ***g*****l h*****q***** **q*pdad*n
POSBE	*gci*****	*****dev** ***g***** m*****q***** ****pd*ds*
D2cDNA	hg*s*****	***e***** *****g* **a**n***** *****.*****
Consensus	--ATVYREWA	PAAQEAQLIG DFNNWNGSNH KMEKD-FGVW SIRISHVNGK
	201	250
RSBEI	*****	***r**g*a* *****.***** **f***** *****
MSBEI	*****	***l*.g*** *****l*** *****.*****
D4cDNA	*****	***hr*d*l* *****.***** **f***** *****
PESBEII	*****r**	***k*sd*** *****k* ***ptr*a* *****y*****
POSBE	*v*****r**	***k**n*** *****k* **a**t**a* *****y*****
D2cDNA	*****	***r*.h*** ***q***** ***t**es** *****l*****
Consensus	PAIPHNSKVK	FRF-HG-GVW VDRIPAWIRY ATVDASKFGA PYDGVHWDPP
	251	300
RSBEI	ac*****	*****.***** *****.***** *****.*****
MSBEI	a*****t*****	**s**a*****.***** k*a***** *****.*****
D4cDNA	sg*****	**r*****.***** r*****.***** *****k*
PESBEII	1*****q*****	*****k*****.***** ss **r*ns**** **d*****e
POSBE	p*****h**y*	*****r*****.***** ss **r*ns**** **d*****k*
D2cDNA	s*****n**	*****v***.***** v***g kl*ag***** p*****cl**
Consensus	-SERYVFKHP	RPKPDAPRI YEAHVGMSGE EPEVSTYREF ADNVLPRIRA

Figure 4

	301		350
RSBEI	*****	*****	*****
MSBEI	*****	*****	*****
D4cDNA	*****	ilcf*	w*****
PESBEII	*****	*****	w*****kp***
POSBE	*****	*****g**	*****.***
D2cDNA	t*****g	*****ds***	*****.***
Consensus	NNYNTVQLMA	IMEHSYYASF	GYHVTN-FFA VSSRSGTPED LKYL-DKAHS
	351		400
RSBEI	*****	*****	n h*****t**
MSBEI	*****	*****	*****a**
D4cDNA	*****	s*m**	n *****t**
PESBEII	n*****	*****	*****s*q*****a**
POSBE	q**v**	*****	*****g s*****a**
D2cDNA	*****	*****i*	*****ah*****yt** k**n***ng*
Consensus	LGLRVLMDVV	HSHASNNVTD	GLNGYDVGQS TQESYFH-GD RGYHKLWDSR
	401		450
RSBEI	*****	*****	*****k***
MSBEI	*****	*****	*****v***
D4cDNA	*****	*****	n *****s*a*
PESBEII	ks.	s*****	*****k*****
POSBE	*****	*****	*****n*****v
D2cDNA	*****	*****	*****v*****n n*****s*n*
Consensus	LFNYANWEVL	RFLLSNLRYW	-DEFMFDFGFR FDGVTSMLYH HHGINMGFTG
	451		500
RSBEI	*****	*****	*****l***
MSBEI	q*****	a*****	*****l***
D4cDNA	*****g***	*****	*****i***
PESBEII	d*n*****e**	*****	**s*v*di**
POSBE	n*****ea*	*****	**n*i**i**
D2cDNA	*****ig***	n***f*****	*****l**
Consensus	NYKEYFSLDT	DVDAVYMMML	ANHLMHK-LP EATVVAEDVS GMPVLCRPVD
	501		550
RSBEI	*****	*****	rk* vq**
MSBEI	*****	*****	g*.ah**
D4cDNA	*****	*****	a.ah**
PESBEII	v*****	k***	*****k***
POSBE	*****	k***	*****n*e**
D2cDNA	l*****q	*****	**e**g*qq*
Consensus	EGGVGFDYRL	AMAIPDRWID	YLKNKDDSEW SMSE-I--TL TNRRYTEKCI
	551		600
RSBEI	*****	*****	t***
MSBEI	*****	*****	t***
D4cDNA	*****	m*****	t***
PESBEII	s*****	*****	e*****ss**
POSBE	*****	*****	c*tml*****
D2cDNA	rqnh**	s***m***	s***c*t***v**
Consensus	AYAESHDQSI	VGDKTIAFL	MDKEMY-GMS DLQPASPTID RGIALQKMIH

Figure 4 (cont..)

	601	650
RSBEI	*****	*****
MSBEI	*****	*****
D4cDNA	*****	*****
PESBEII	*****	*****
POSBE	*f*****	*****
D2cDNA	*****S	**k****
Consensus	FITMALGGDG	YLNFMGNEFG
	HPEWIDFPRE	GNNWSYDKCR
		-RQWSLVDTD
	651	700
RSBEI	*****	e
MSBEI	*****	r
D4cDNA	*****	*****
PESBEII	*****	*r***l***
POSBE	*****	*r***s***
D2cDNA	.....	v***vdtps**
Consensus	HLRYKYMNAF	DQAMNALD-K
	FSFLSSSKQI	VSDMNEE-KV
		IVFERGDLVF
	701	750
RSBEI	*****n***	k*****
MSBEI	*****k***	*****
D4cDNA	*****s***	*****
PESBEII	*****en**	*****
POSBE	*****kn**	*****
D2cDNA	.*thlrsgc*	*p.....s**
Consensus	VFNFH-P-KTY	EGYKVGCDLP
	GKYRVALDSD	AL-FGGHGRV
		GHDVDHFTSP
	751	800
RSBEI	**m*****	*****
MSBEI	*****	*****
D4cDNA	*****	*****
PESBEII	*****	*****
POSBE	*****	*****
D2cDNA	ifcc*lfkge	*
Consensus	EG-PGVPETN	FNNRP----
		-----NSFKV
		LSPPRTCVAY
	801	850
RSBEI	*...*****dr	**l*rg**va
MSBEI	*...*****ag	agr*lhak*e
D4cDNA	*...*****ka	*kpkde***
PESBEII	*...*****q	**snnpnlg*
POSBE	*yqqp*sr*v	trnlkirylq
D2cDNA	.....	sv***tna*q
Consensus	Y---RVDER-	EE-R--GAAS
	-GKT-PA-YI	DV-ATR----
		-SGE--SG--
	851	876
RSBEI	kg***d*cg*	**mk***r**
MSBEI	edk*atagg*	**wk*arqp*
D4cDNA	ka*tgg**ss*	**in***g*p
PESBEII	r*e*ns**av	dagi*kvere
POSBE	r*tr*lk*sl	vvgdn*stnist*...
D2cDNA	.....	.....
Consensus	--SEK-DD-K	KG--FVF-SS
		D-D-K-

Figure 4 (cont..)

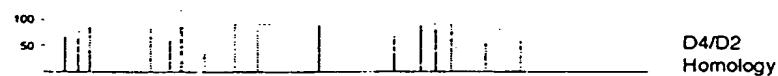
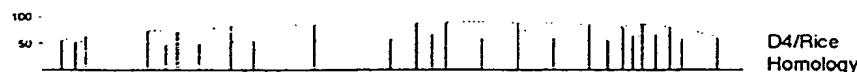
Wheat-D2



Wheat-D4



Rice

D2/Rice  
HomologyD4/D2  
HomologyD4/Rice  
Homology

0 1000 2000 3000 4000 5000 6000 7000 8000 9000

Exon



Intron



Homology of corresponding exons

and introns

FIGURE 5

5' TCCCGCTGCGCCAAAGAGACTACACCATGGCAACAGCTGAAGATGGTGGCGACCT 5'  
 3' AGGGCACAGACGGGTTCTCTGATGGTACCGTCTGACTTACCAACCGCTGG 3'

DNA

<p>possible reading frames</p>	<p>S R V C A K R L H H G N S * R W C W R P</p>	<p>P V S A P R D Y T M A T A E D G V G D L</p>	<p>P C L R Q E T T P W Q Q L K M V L A T F</p>	<p>true N- terminal sequence for BE-1 (Morell et al, 1997)</p>
--	--	--	--	--

Figure 6

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A 1 2 3 4 5 6 7 8 9 10 11 12

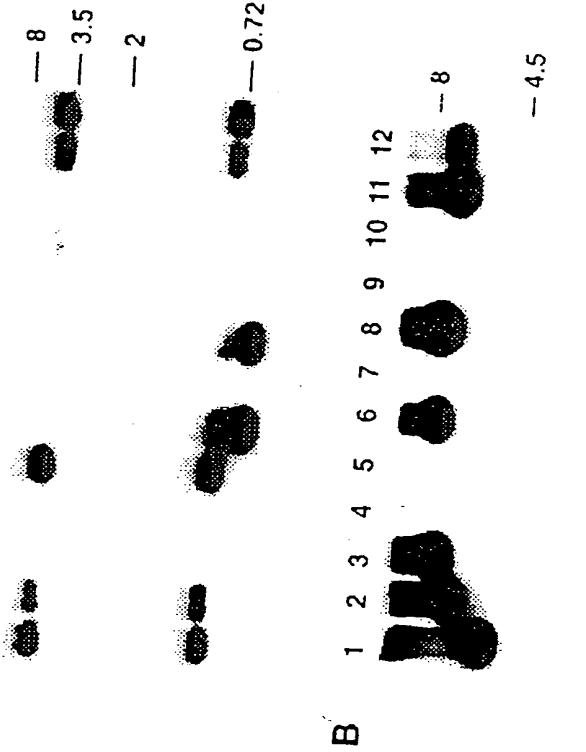


FIGURE 7

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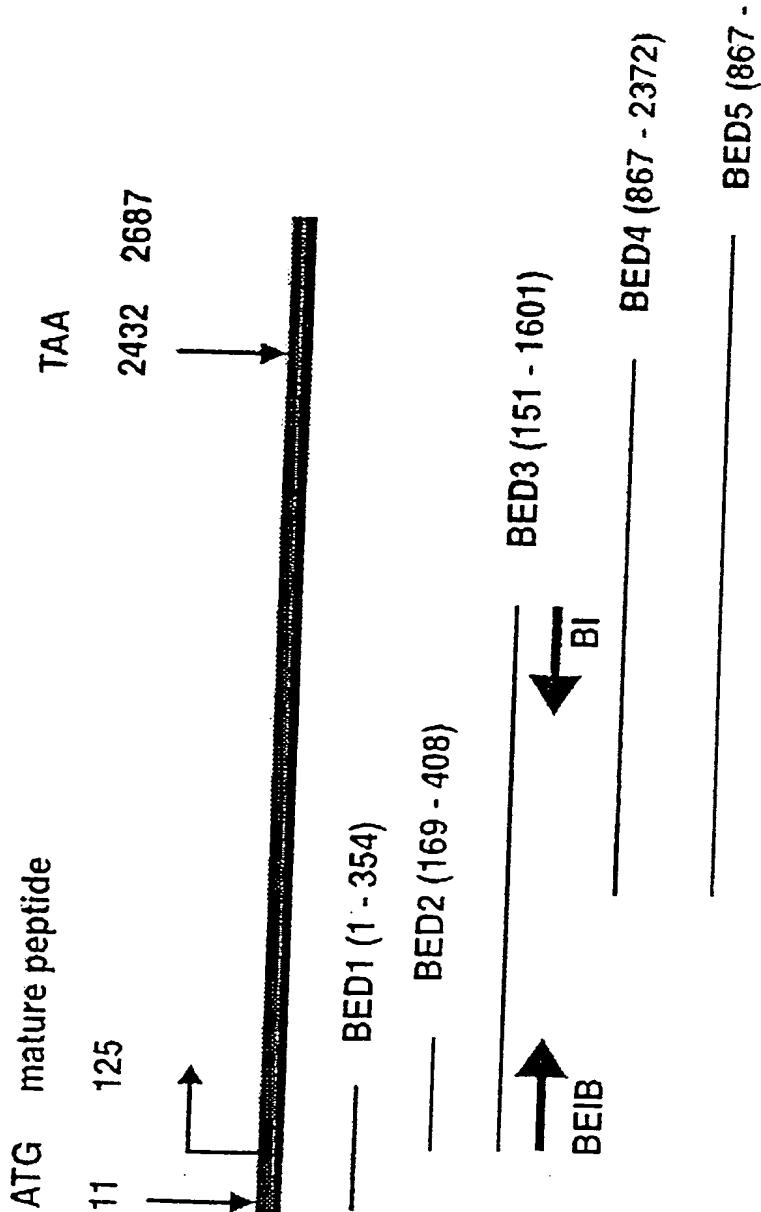


FIGURE 8

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## Expression of Starch Biosynthetic Genes

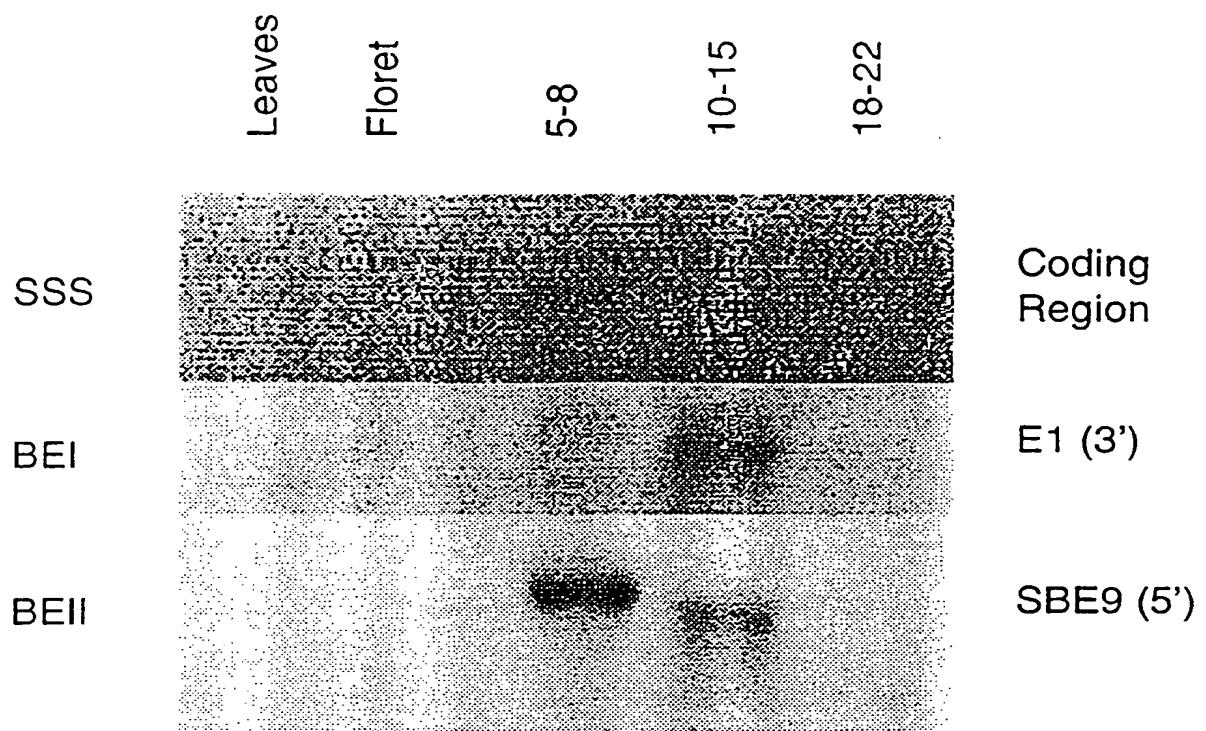


FIGURE 9A

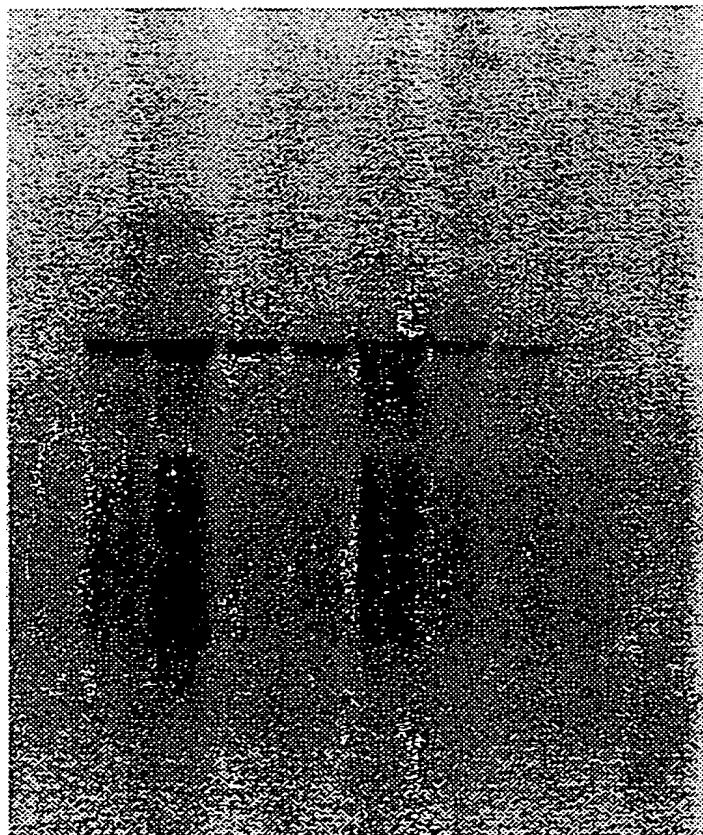
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4 6 8 10 12 15 18 21 25 31



FIGURE 9B

4 6 8 10 12 15 18 21 25 31



← 2.9 kb

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4 6 8 10 12 15 18 21 25

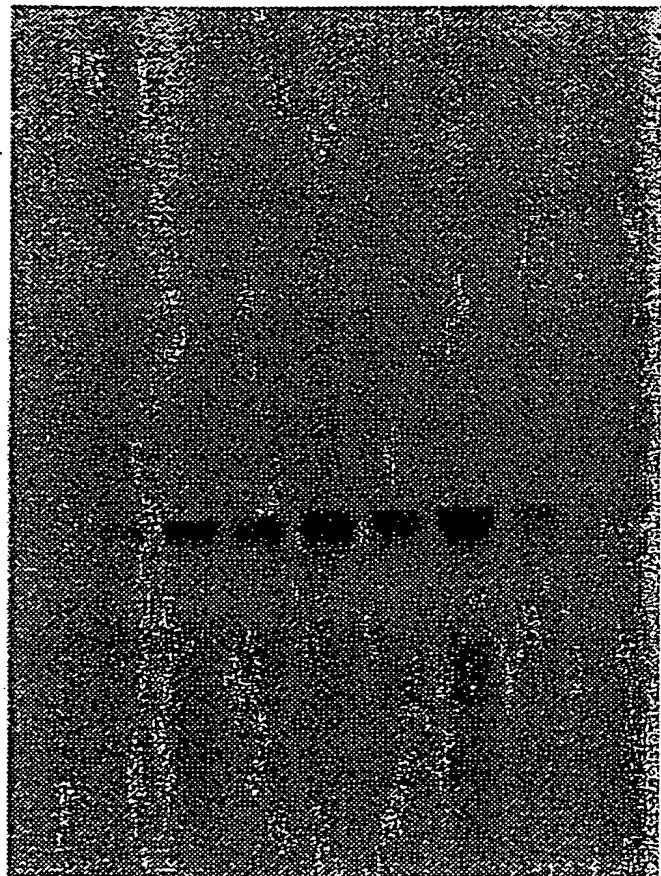


FIGURE 9D

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4 6 8 10 12 15 18 21 25

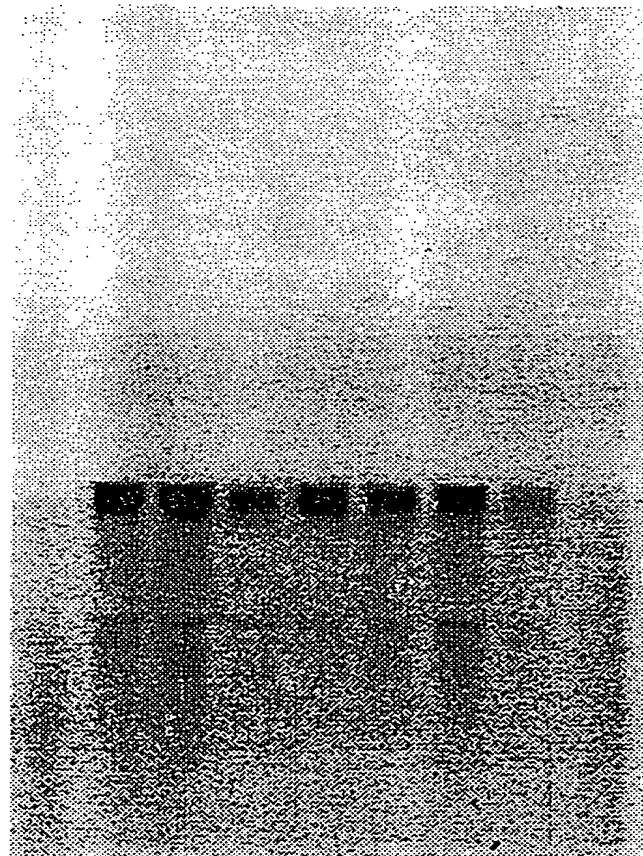


FIGURE 9E

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4 6 8 10 12 15 18 21 25

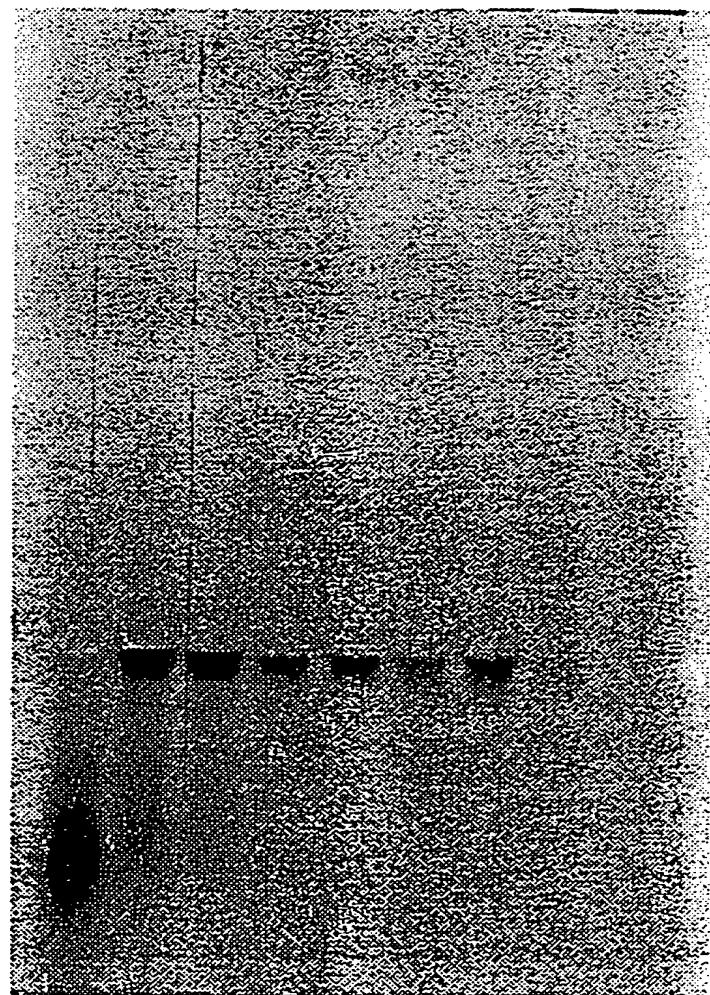


FIGURE 9F

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4 6 8 10 12 15 18 21 25

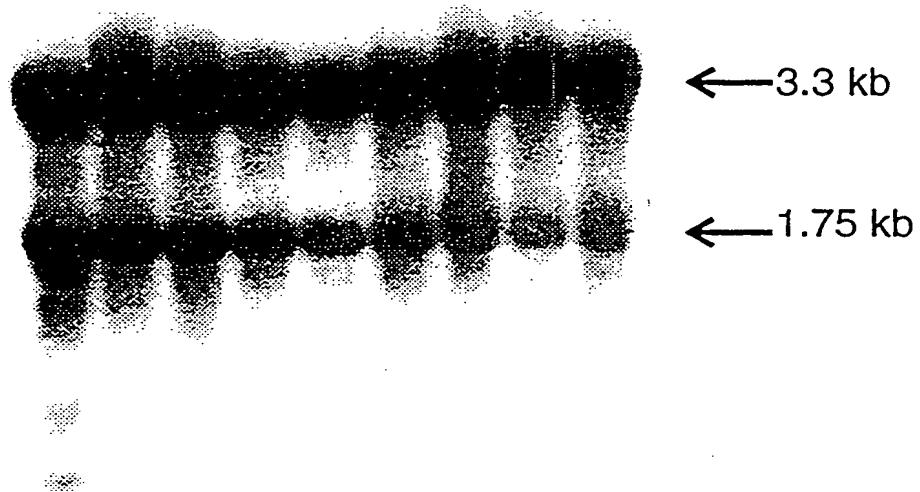


FIGURE 9G

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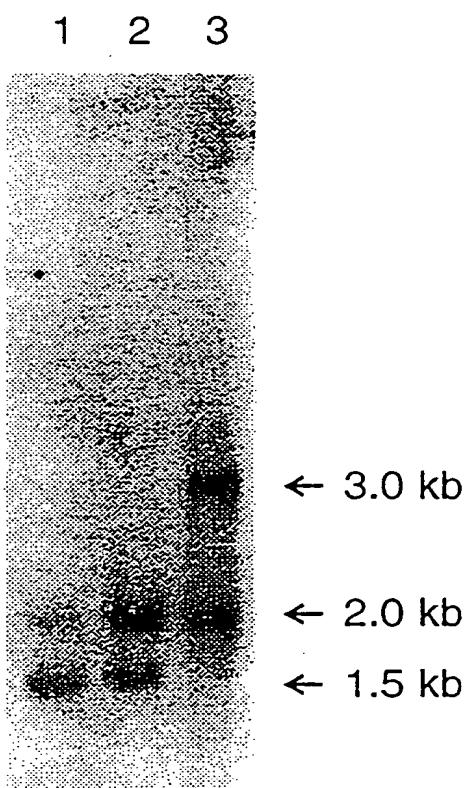


FIGURE 9H

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DOTPLOT of: d10838.pnt Density: 12614.77 February 18, 1997 11:43

COMPARE Window: 21 Stringency: 14.0 Points: 20,788

sr427.res ck: 6,362, 1 to 11,099

d10838.em\_pl ck: 3,071, 1 to 11,700

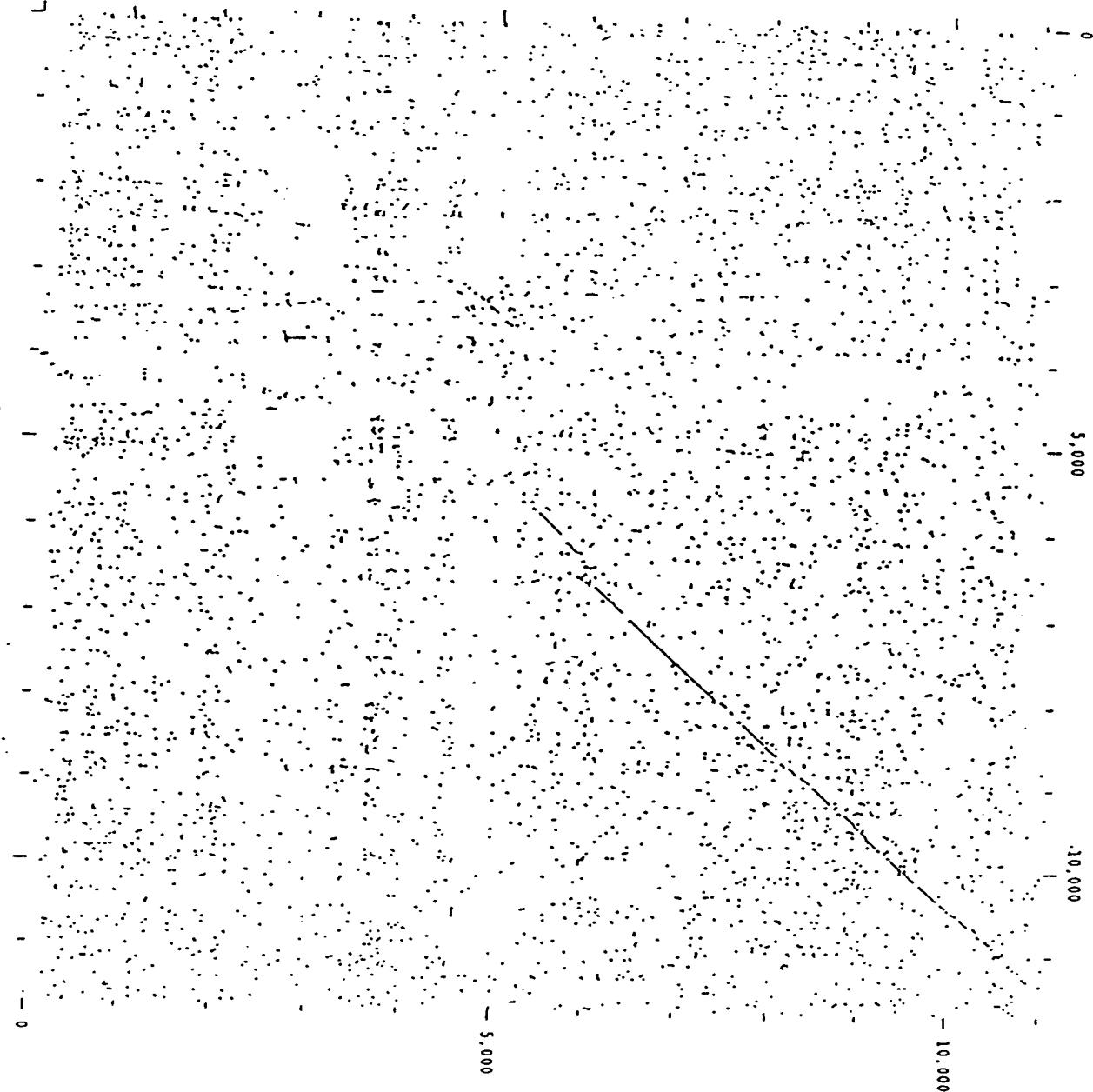


Figure 10

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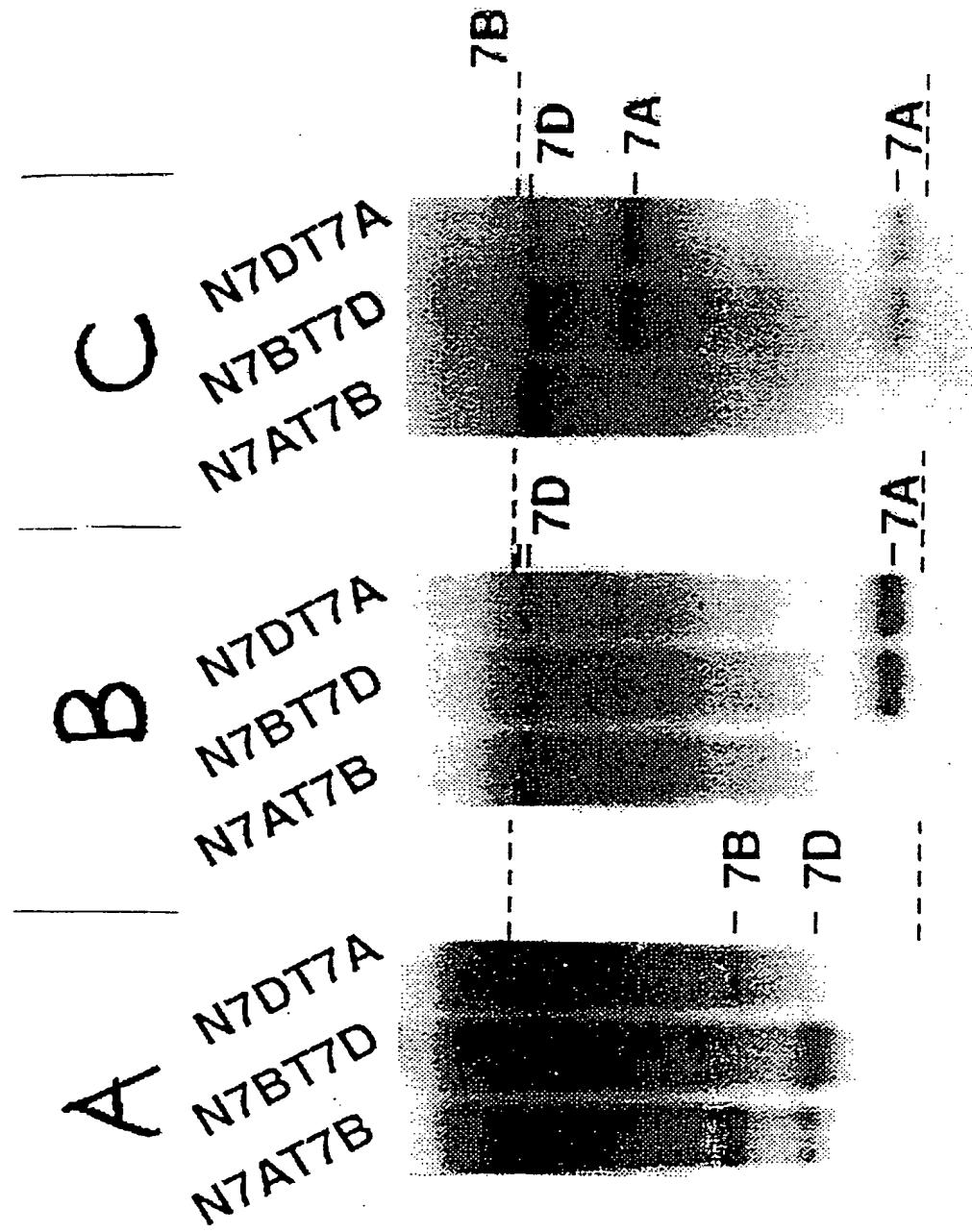


FIGURE 11

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Genomic Clones from *T.tauschii*  
for SBE II.

BamH I    EcoRI

F1    F2    F3    F4

kb  
8.0  
4.1  
0.7

FIGURE 12

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## N-terminal sequences of cereal starch branching enzymes

Protein	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1	1	2	2
A										0	1	2	3	4	5	6	7	8	9
WBE-I <sup>d</sup>	V	S	A	P	R	D	Y	T	M	A	T	A	E	D	G	V			
MAIZE	A	T	V	Q	E	D	K	T	M	A	T	M	A	T	K	G	D	V	
BEI <sup>c</sup>																			
RICEBEI <sup>b</sup>	A	T	A	R	K	N	K	T	M	V	T	V	V	E	E	V			
WBE-II <sup>d</sup>	A	A	S	P	G	K	-	V	L	V	P	D	G	E	S	D	G	M	P
MAIZE	A	A	A	A	R	K	A	V	M	V	P	E	G	N	D	G	L	A	S
BEII <sup>e</sup>																			

<sup>a</sup> N-terminal amino acid of the mature polypeptide. <sup>b</sup> Kawasaki *et al.* (1993), <sup>c</sup> Baba *et al.* (1991),<sup>d</sup> Mizuno *et al.* (1993), <sup>e</sup> Fisher *et al.* (1993)

Residues in the wheat sequences showing identity with the respective maize or rice branching enzyme isoforms are highlighted in bold text.

Figure 13a

TTCCCTTTTTTTCTTGGGNGGGGATGCCC AGTGGATGNTGTTCCCAATGAAATT 1 60  
 AAGGGAAAAAAAGAAAACCNCACCGGACACCTACACAGGGTTACTTAAA

a F P F F F G ? G M A C W M ? F P N E F -  
 b S L F F S L G G G W P V G ? C S P M N F -  
 c P F F F L W ? G D G L L D ? V P Q \* I S -

CCATGGAGTGGAGAGAGATAGTTGGATNAGGGATCGCGNTTCCNGGAACGTGATTTTTC 61 120  
 GGTACCTCACTCTCTATCAACCTANTCCCTAGGCNAAGGNCCGTGACATAAAAAG

a P W S E R D S W ? R D R ? S ? N C I F F -  
 b H G V R E I V G ? G I A ? P G T V F F S -  
 c M E \* E R \* L D ? G S R F ? E L Y F F P -

CCCGNGGGGGAAATGGCGTTAGTGTCAACCCAGGCCCTGGTGTACACGGCTTGATC 121 180  
 GGGNGCCGCCCTTACCGCAATCACAGNTGGTCCGGGACCAATGGTCCCGAAACTAG

a ~~A~~ P ? G G N G V S V ? P G P G V T T A L I -  
 b P A G E M A L V S T Q A L V L P R L \* S -  
 c ? R G K W R \* C ? P R P W C Y H G F D H -

ATTCTTCGTTTCATTCTGATATATATTTCTCATCTCTCTCTCTCTCTCTCTCTGAA 181 240  
 TAAGAACCAAAGTAAGACTATATATAAAAGAGTAAGAAAAGAAGGACAAGAACGACATT

a I L R F I L I Y I F S F F F F L F L L \* -  
 b F F V S F \* Y I F S H S F S S C S C C N -  
 c S S F H S D I Y F L I L F L P V L A V T -

CCTCAAGTTGTGGGTTTTTCACTATTGACTCATCTTGCATTTCAGGOGOOGTOC 241 300  
 GAOGTCAACAOOGCAAAAAGTGTATAACATCACTAGTAGAACGTAAAACGTCCGGCCAGG

a L Q V V A F F H Y C S H P C I L Q A P S -  
 b C K L W R F F T I V V I L A F C R R R P -  
 c A S C G V F S L L \* S S L H F A G A V L -

TGAGGGGGGGGGGGCTCTCAGGGAAAGGTCTGGTGCCTGACGGGGAGAGNGAOGACTTGG 301 360  
 ACTGGGGGGGGGGAGAGGTCCCTCCAGGACCAAGGACTGCCGCTCTGCTGAAACC

a \* A A R P L Q G R S W C L T A R ? T T W -  
 b E P R G L S R E G P G A \* R R E ? R L G -  
 c S R A A S P G K V L V P D G E ? D D L A -

CAAGTCCGGCGCAACCTGAAGAATTACAGGTACACACACTCGTCCGGTAAATCTTCATA 361 420  
 GTTCAGGGGGGGCTTGGACTCTCTAAATGTCCATGTGTTGAGGACACGGGCACTTGAAGGT

a Q V R R N L K N Y R Y T H S C R \* I F I -  
 b K S G A T \* R I T G T H T R A G K S S Y -  
 c S P A Q P E E L Q V H T L V P V N L H T -

CAATCGTTATTCACTTACCAAATGCCGGATGAAACCAACCAAGGATGCCGTAGGTTTCA 421 480  
 GTTAGCAATAAGTGAATGGTTACGGCCTACTTTGGTTGGTGCCTACCCAGTCACAAAGCT

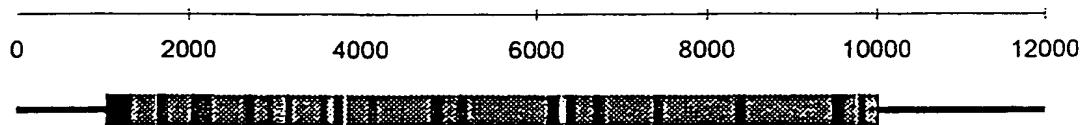
a Q S I F T Y O M P D E T N H G C V R F R -  
 b N R Y S L T K C R M K P T T D A S G F E -

Figure 13b

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*Branching Enzyme-II Genes*

## Intron/Exon structure of wheat BE-II



## Schematic Diagram of a cDNA for BE-II

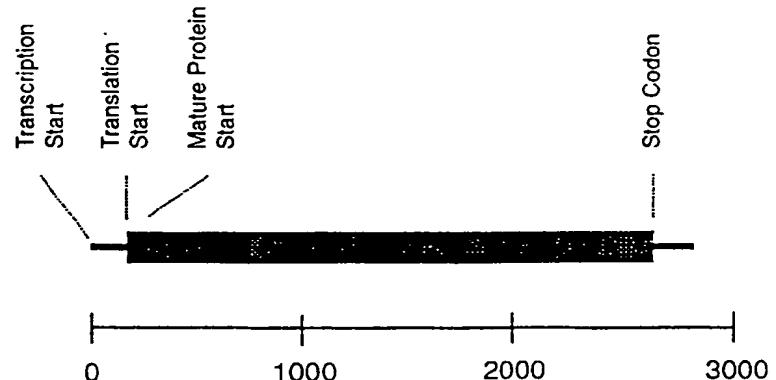


FIGURE 14

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Wheat DNA probed with the  
5' conserved sequence of SBE II.

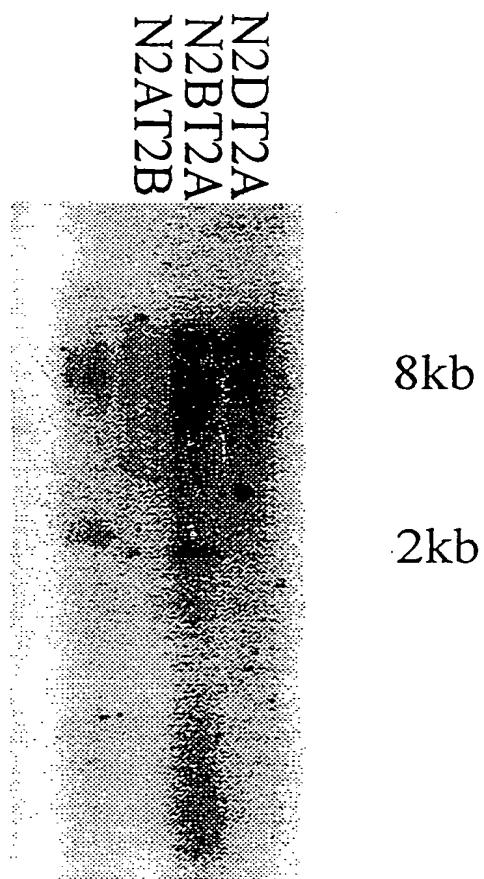


FIGURE 15

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COMPARISON OF N-TERMINAL SEQUENCES  
OF SOLUBLE STARCH SYNTHASE

GRYVAELSREGPAARP

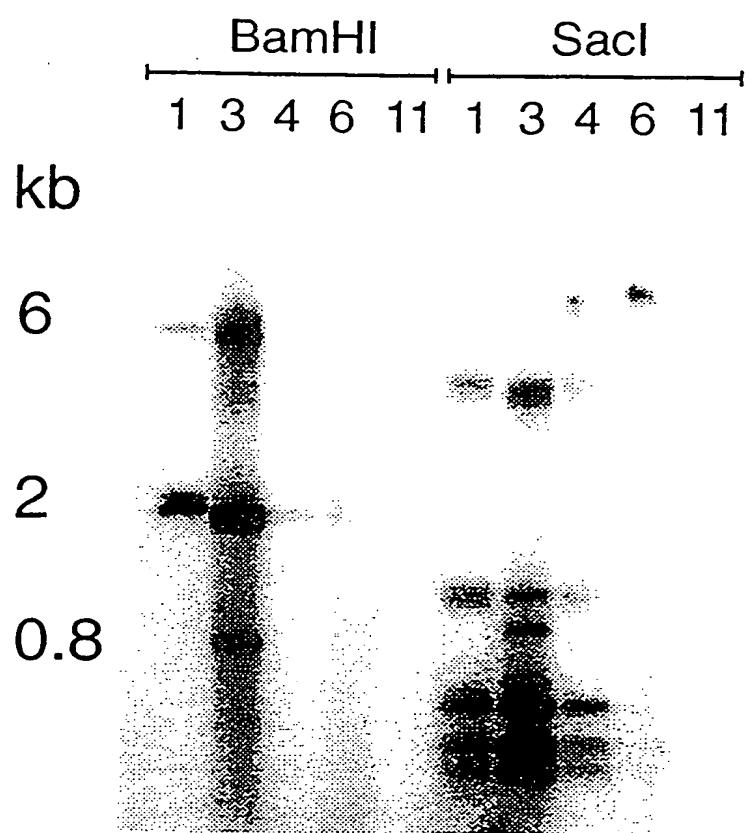
Deduced from wheat cDNA

GPYVAELSPEGPAAPP

Wheat N-terminal

Figure 16

# Soluble Starch Synthase Genomic Clones

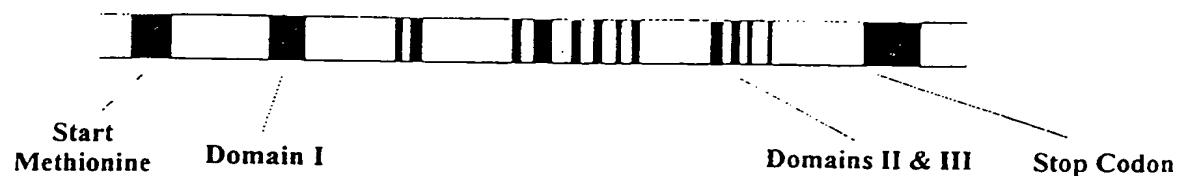


Probed with SM-2 full length cDNA

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**INTRON EXON STRUCTURE - Wheat SSI**

Rice SSI genomic DNA



Wheat SSI genomic DNA

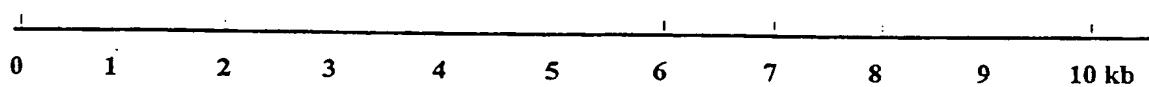


FIGURE 18

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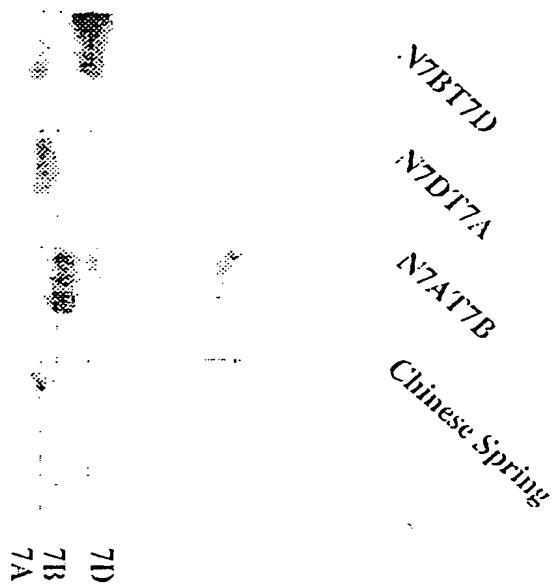


FIGURE 19

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Figure 20a

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## Comparison of Wheat Debranching Enzyme-I (WDBE-I) PCR fragment with maize *Sugary-1* DNA sequence

SUGARY.DNA	1098	1107	1117	1127	1137	1147	1157
	TGAGGTGATCATGGATGTTGTTCTTCATACAGCTAAGGTAAATGAGGAGCCCAAT						
WHEAT1.DNA	...-3	6	16	26	36	46	56
FILE NAME	1158	1167	1177	1187	1197	1207	1217
SUGARY.DNA	ATTATCCTTTAGGGGTTAGATGATGTTACATACTACATCTACCTAAGGGAGAGTT						
WHEAT1.DNA	57	66	76	86	96	106	116
FILE NAME	1218	1227	1237	1247	1257	1267	1277
SUGARY.DNA	TTATAATTATCTGGTTGGAAATTACCTTCATTGTAATTCACTCCTGTTGTTGTTGATT						
WHEAT1.DNA	117	126	136	146	156	166	176
FILE NAME	1278	1287	1297	1307	1317	1327	1337
SUGARY.DNA	TATAGTGATGTTGATCTTGATGATCTGGTAAATGAAATGATGTTGATGTTGTTGTTGAA						
WHEAT1.DNA	177	186	196	206	216	226	236
FILE NAME	1338	1347	1357				
SUGARY.DNA	DCTTCATCATCTACT-G...						

MATCHING PERCENTAGE	
TOTAL WINDOW	84%
ALIGNMENT WINDOW	86%

Figure 20b

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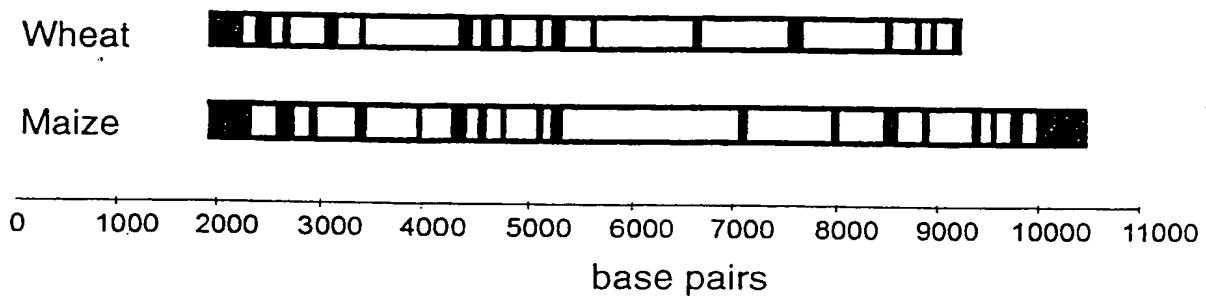
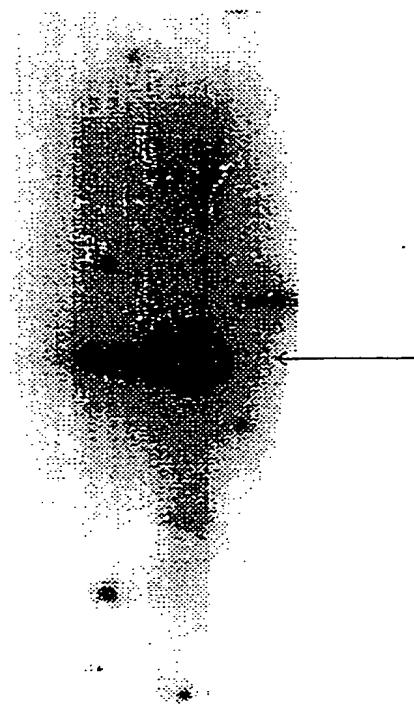


FIGURE 20C

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Southern blot of *T. tauschii*  
Genomic DNA

1X 3X



BamHI Digest

*T. tauschii* Genomic DNA Probed  
With The Wheat Debranching Enzyme  
PCR Product

FIGURE 21A

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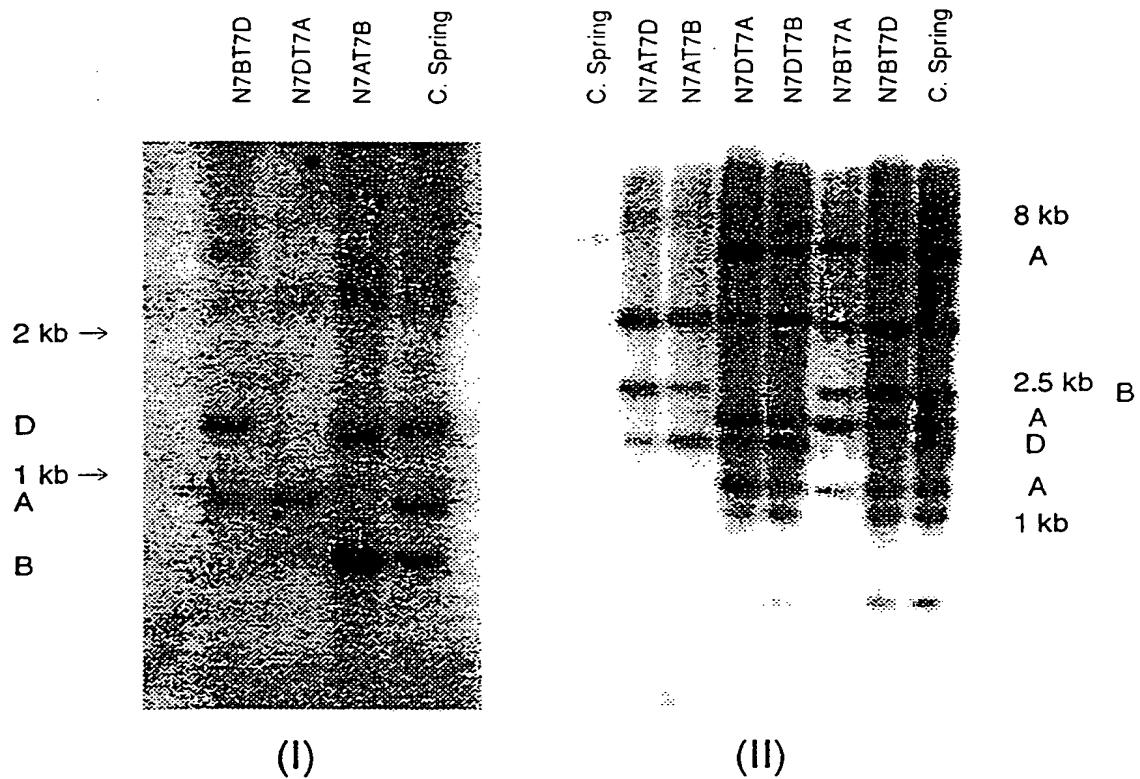


FIGURE 21B

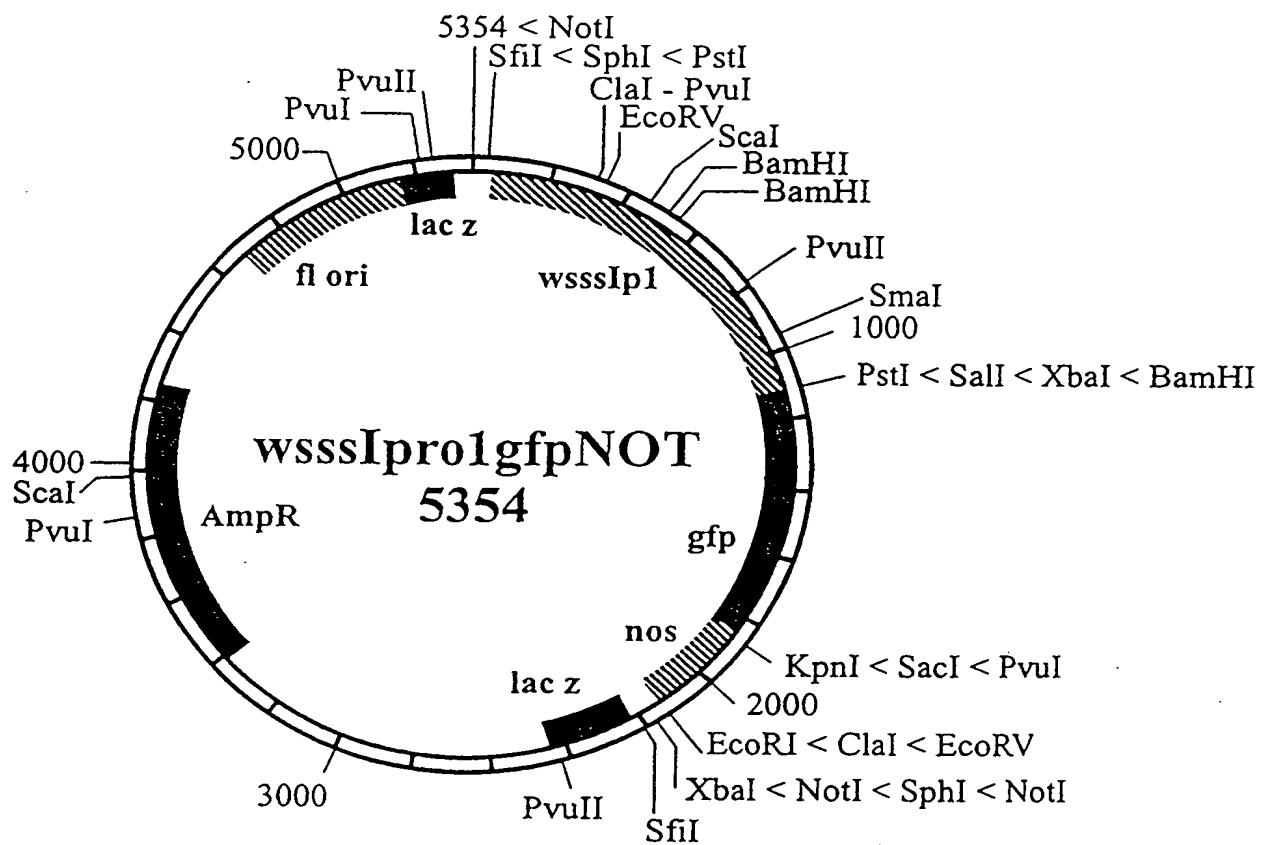


FIGURE 22A

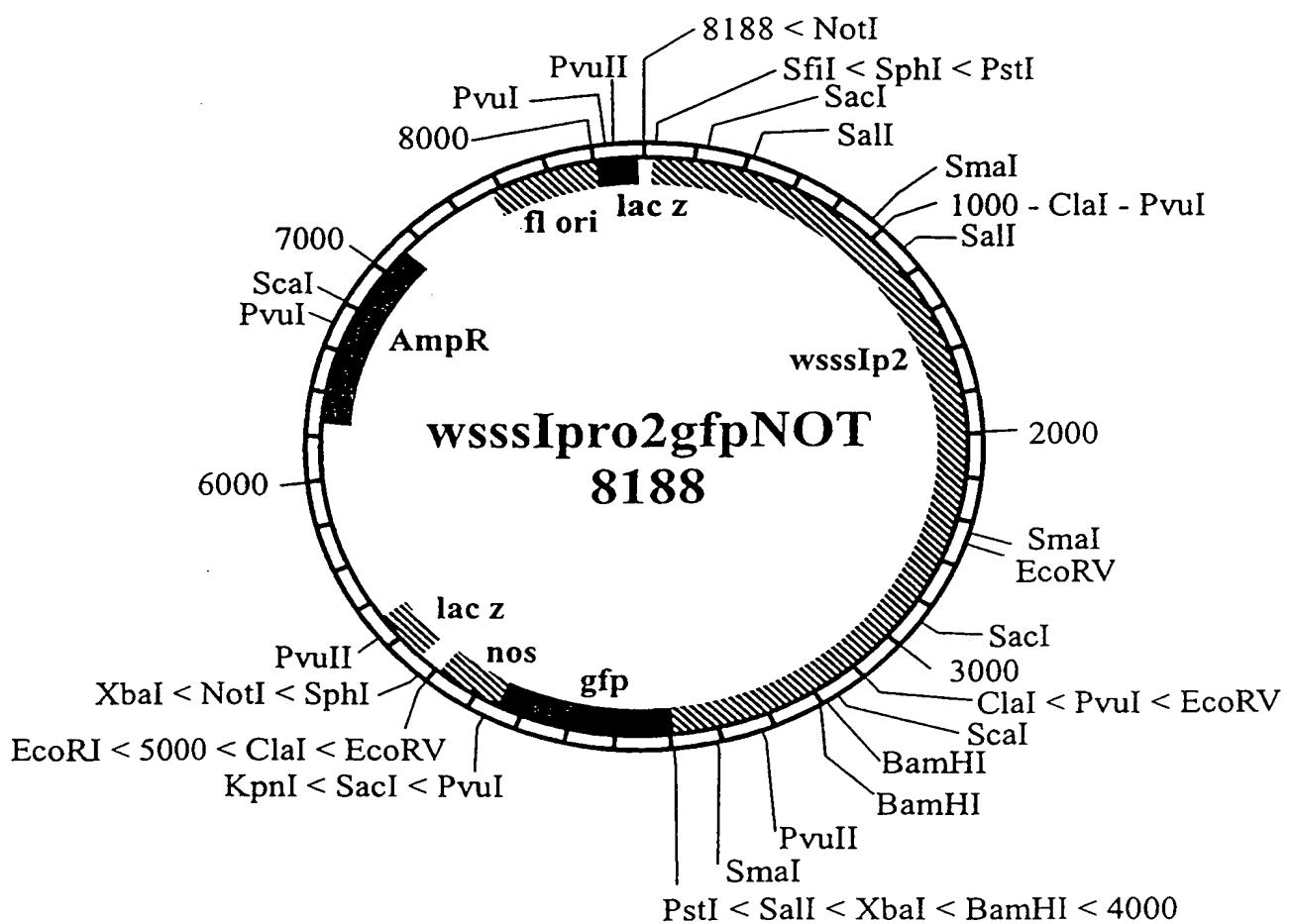


FIGURE 22B

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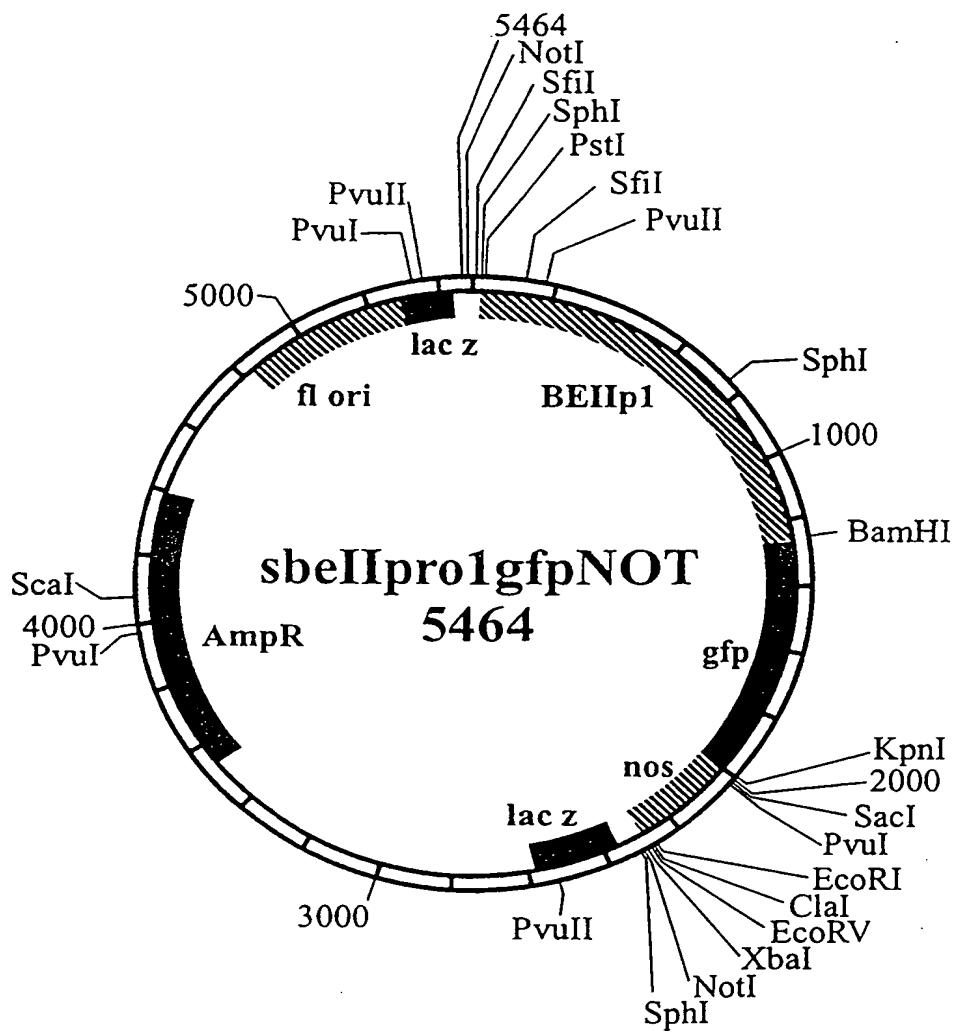


FIGURE 22C

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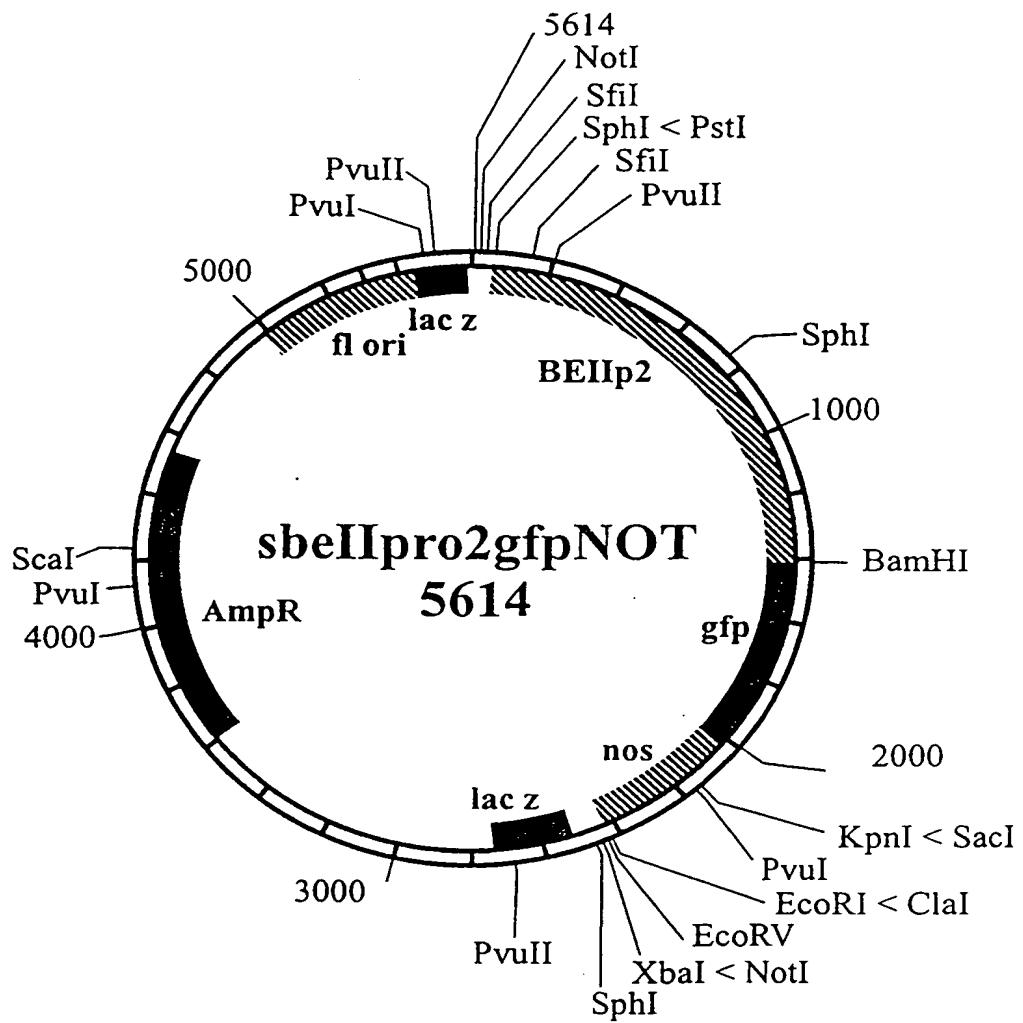
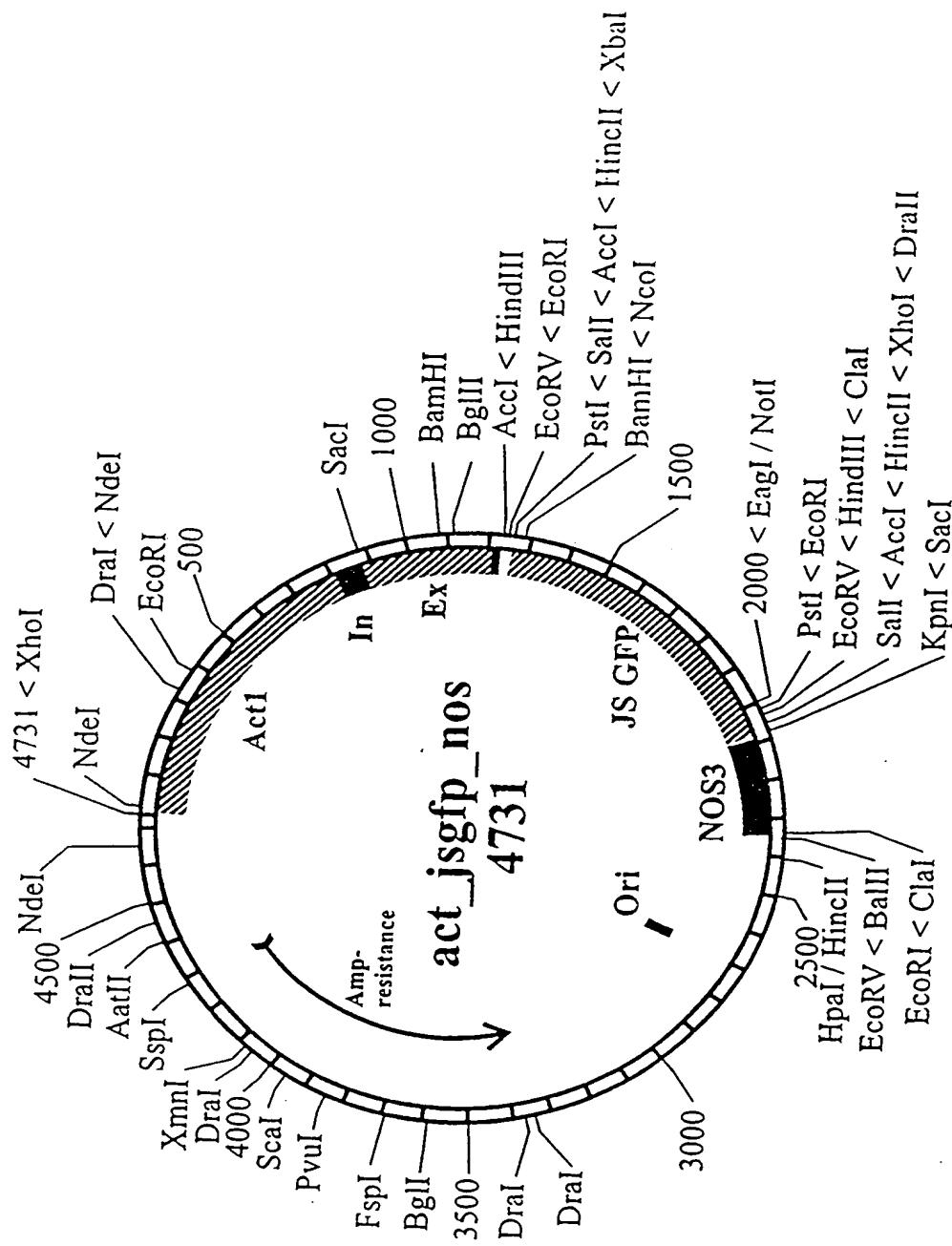


FIGURE 22D



**Figure 22E**  
SUBSTITUTE SHEET (Rule 26) (RO/AU)

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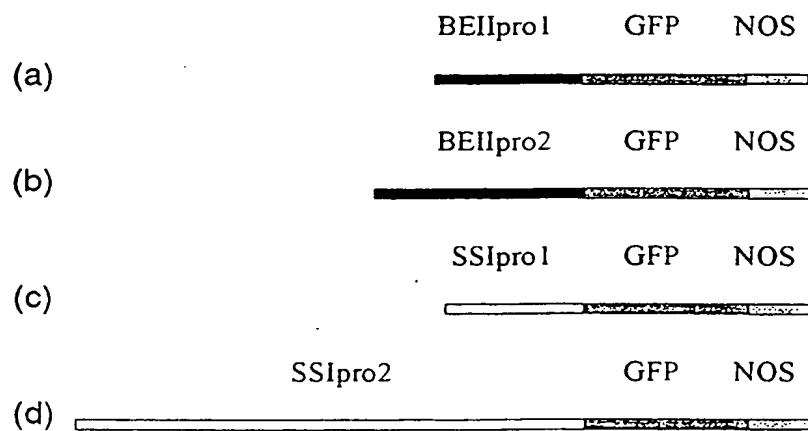
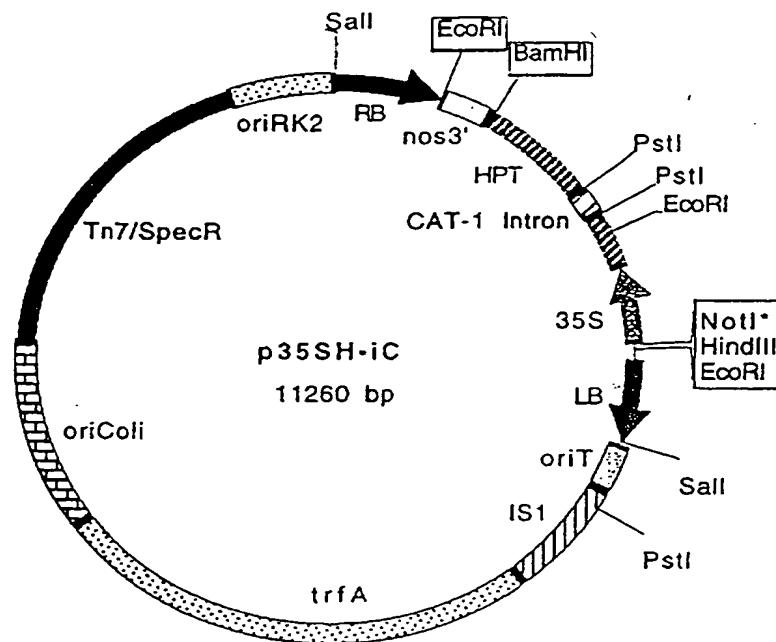


FIGURE 23

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Primer Set	Key	Forward Primer	Forward Primer Sequence
1	E01'/E02	WBE2E1F	CGT CGC TGC TCC TCA GGA AG
2	E01/E02	sr854.1180F	CTG GCT GAC TCA ATC ACT ACG
3	E02/E03	WBE2E2F	CGC AAC CTG AAG AAT TAC AG
4	E03/E04	WBE2E3F	ATT TTC GGA GCC ATC TTG AC
5	E04/E05	WBE2E4F	TCG TGG TTA TGA AAA GCT TGG
6	E05/E06	sr913F	ATC ACT TAC CGA GAA TGG G
7	E05/I05	sr913F	ATC ACT TAC CGA GAA TGG G
8	E06/E07	WBE2E6F	ACA ATT GGA ATC CAA ATG CA
9	E07/E08	WBE2E7F	AGC TAT TCC TCA TGG CTC AC
10	E08/E09	WBE2E8F	TGC AGG CTC CAG GTG AAA TA
11	E10/E11	da5.seq	GGC TTG GAT ACA ATG CAG TGC
12	E12/E13	da151.seq	TTG ACG GCT TGA ATG GTT TC
13	E17/E18	WBE2E17F	TTT AGG TGG TGA AGG CTA TCT
14	E18/E19	sr860R	AAT GGA TAG ATT TTC CAA GAG G
15	E19_3'	WBE2-2395F	AGC AGA ACT GCG GTC GTG TA

Reverse Primer	Reverse Primer Sequence	Temp	bp
WBE2E2R	CAG GAC CTT CCC TGG AGA GG	57.4	401
WSBE9E2R	GGC ACG AGT GTG TGT ACC TGT A	57.7	601
sr866F	TAT CTT CAG GTA TCT ACA GC	49.8	309
WBE2E4R2	ATG CTT CCA ATC CAC CTT CA	-	>450
WBE2E5R	GAG CCC ATT CTC GGT AAG TGA	50.5	234
WBE2E6R	CTG CAT TTG GAT TCC AAT TG	49.9	232
WBE2I5R	CAG TAA GCT AGT TGG TGA ATA	46.6	106
WBE2E7R	GGG AGG AAA ATC TCC CAA AC	51.0	402
sr915F	CCA TTG AAA GGT ATT TCA CC	51.1	203
sr912F	TAA CTT ATT GAC ATA CCG G	48.4	439
WBE2E11R	CTG GAG TTC CAA AAC GGC TAC	51.2	289
WBE2E13R	ATT CTT CAA GCC ACC ATC TC	51.6	244
WBE2E18R	TAT TGT TAT TTC CAG GGG AGA	50.2	258
da23.seq	TGC TGC ATT GCC TGA TCG AA	50.4	~295
WBE2-2634R	AAC ACC CAG GCC CGT CCA TT	57.2	240

Figure 24

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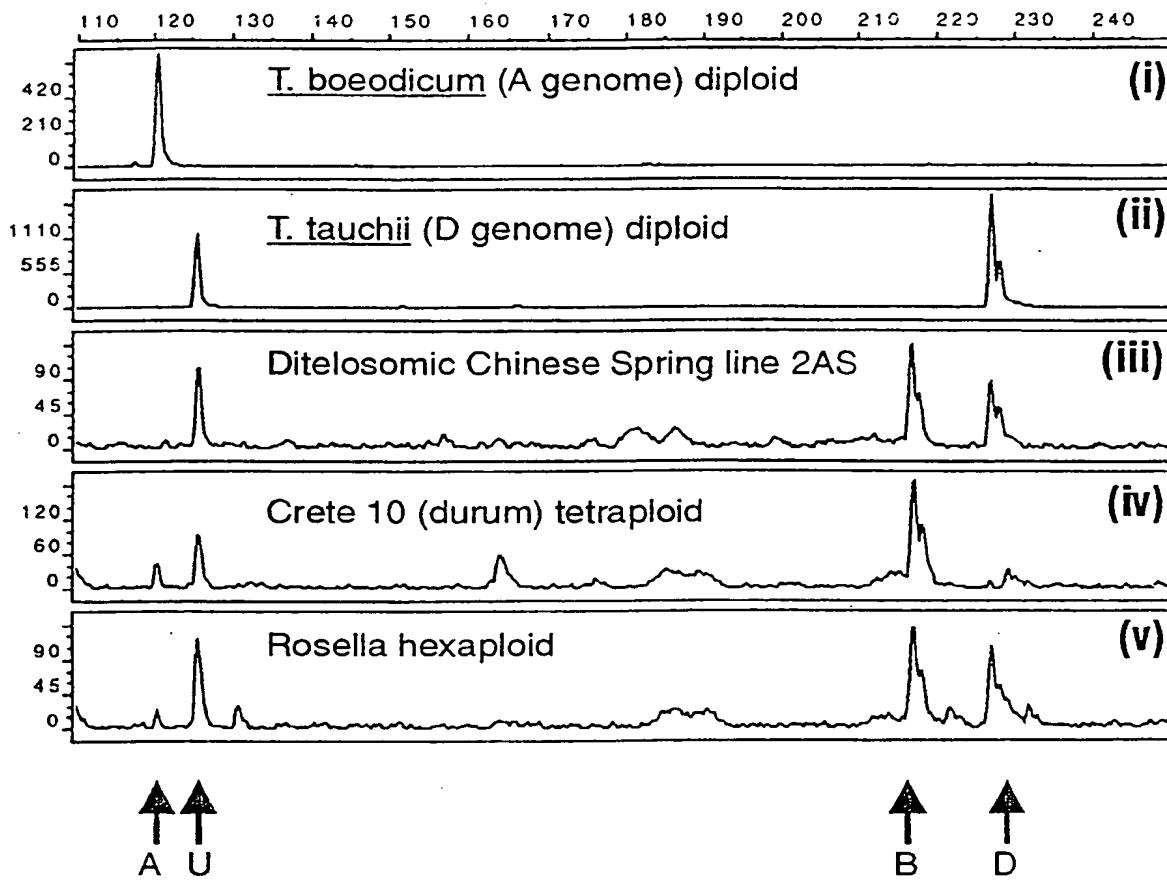
SBE II Intron 5 primer set - digested with Dde1

FIGURE 25

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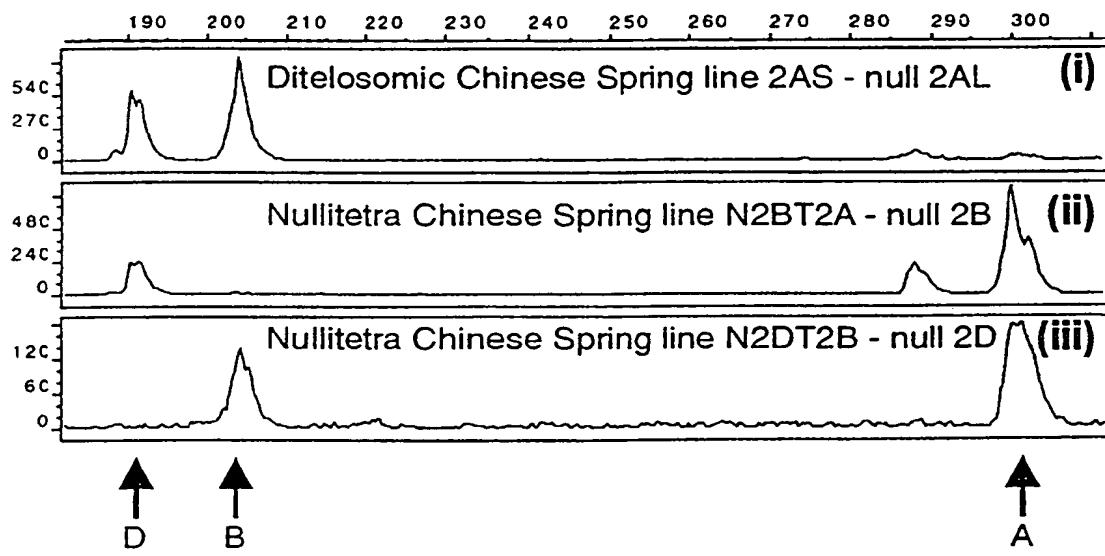
SBE II Intron 10 primer set - digested with Dde1

FIGURE 26

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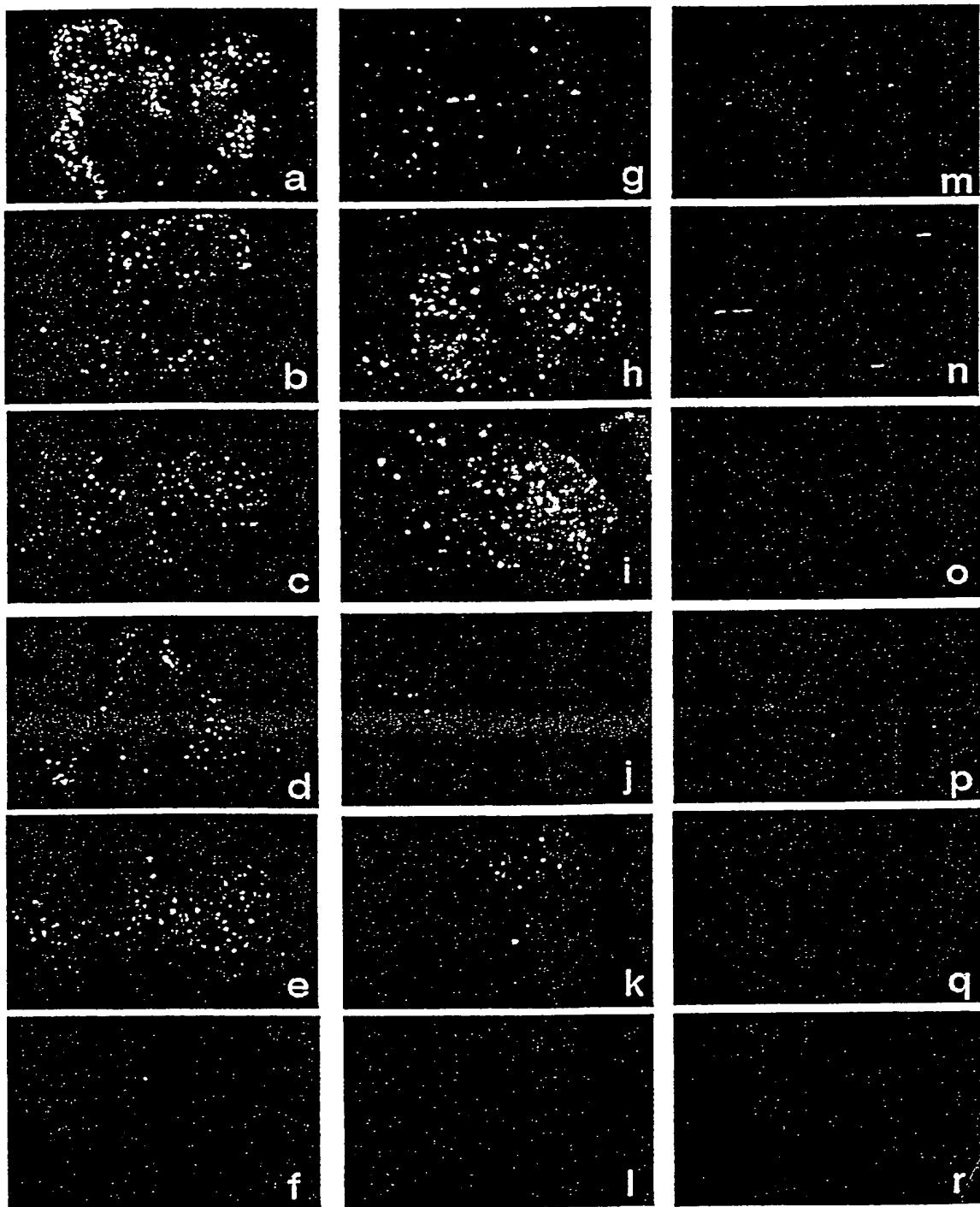


FIGURE 27